UNIVERSITY OF ARKANSAS

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COTTON WILT STUDIES

1. RELATION OF SOIL TEMPERATURE TO THE DEVELOPMENT OF COTTON WILT

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COTTON WILT STUDIES

1. The Relation of Soil Temperature to the Development of Cotton Wilt

V. H. YOUNG[1]

The prevalence of cotton wilt in most of the light sandy soils of the cotton belt of the United States and the steady loss of an appreciable percentage of the cotton crop every year from this disease entitles it to rank of major importance among plant diseases. Although it is now some thirty-six years since this disease was first described by Atkinson (1892) from Alabama and Arkansas, very little is yet known concerning the conditions governing its severity. Practically every worker who has studied cotton wilt has noted that it occurs most frequently in the light sandy, well-drained soils of the Coastal Plains regions and is largely absent from the clay soils of the Piedmont. It has also been commonly noted that wet spots in a field are not subject to cotton wilt, that the disease commonly does not appear for several weeks after planting and that the presence of root-knot or nematode disease greatly increases the severity of the attack.

The great desideratum for the control of a plant disease of this type is, of course, some simple soil treatment such as the application of stable manure, commercial fertilizer or lime which will give commercial control of the disease, and many field tests of this nature have been made with cotton wilt. Unfortunately the results obtained have been largely negative in their character and tend to cast a large measure of doubt on the positive results reported in a few cases.

Another type of control for the cotton wilt disease, namely, the development of wilt resistant varieties, has been fairly successful. Unfortunately the varieties so far developed are far from being satisfactory, especially under boll weevil conditions where early maturity is an important consideration, and on this account many growers prefer to employ the early maturing but highly susceptible varieties of cotton such as Trice and Delfos.

The writer is of the opinion that further progress with the control of cotton wilt is dependent upon much more exact knowledge concerning the effects of soil environment upon the cotton plant and upon the development of cotton wilt. Furthermore, it seems highly desirable that a study be made concerning the chemical and physical bases of wilt-resistance in cotton and their method of inheritance. The present study deals with one

[1]Sam E. Poe and John R. Large, graduate students, rendered valuable assistance in the course of this work.

phase of this problem, namely, the relation of soil temperature to the development of cotton wilt under controlled conditions in the greenhouse. The results of certain preliminary observations in the field dealing with the effects of the same factor under natural conditions are also included. Other phases of the problem such as the relation of soil moisture and certain other soil factors to the development of cotton wilt and a further study of the physiology of the pathogene, are now under way.

The literature dealing with cotton wilt has recently been reviewed in detail by Rosen (1926), Fahmy (1927) and Neal (1927), and for this reason it is here presented principally from the point of view of the effects of environment upon the development of cotton wilt. The writer has also desired to present a comparatively complete summary of our knowledge concerning the effects of soil factors upon the cotton plant itself and upon the development of certain other diseases of the cotton plant, and he, therefore, has included a separate review of certain other work along this line. The work dealing with the effects of soil temperature upon the development of several of the Fusarium wilts and upon certain other soil-borne plant diseases has recently been reviewed in detail by Jones, Johnson and Dickson (1926), and for this reason no formal review of this part of the literature is included and only such mention of it is made as is directly related to the problem at hand.

REVIEW OF THE LITERATURE DEALING WITH COTTON WILT

Atkinson (1892) called attention to a disease known commonly to planters in Alabama as "frenching". Cotton plants affected with this trouble had been noted by him from several stations in Alabama and from Arkansas. The disease was first noted on plants about 12 inches high and nearly ready to bloom, but was later noted on plants in the cotyledon stage a few days after emergence from the soil. The symptoms, including yellowing, drying out and death of portions of the leaves, defoliation, dwarfing and partial recovery were noted. Atkinson noted the darkening of the vascular system and pictured the mycelium of a Fusarium in the vessels of the xylem of cotton affected with "frenching" which he was able to isolate by appropriate aseptic methods, and for which he proposed the name *Fusarium vasinfectum*. Atkinson further noted the apparent tendency of the pathogene to enter near the surface through wounds caused by the soreshin fungus and called attention to an aggravated type of "frenching" associated with root-knot caused by the nematode *Heterodera radicicola*. Atkinson found that "frenching" or cotton wilt was especially prevalent and destructive in sandy soils, in gray lime lands and in "gunpowder" land, which be-

comes powdered and loose in dry weather. He also noted that it was less prevalent in clay soils.

Smith (1899) studied the Fusarium wilts of cotton, cowpea and watermelon, and decided that the internal parasite causing these diseases was related to a pyrenomycetous fungus for which he proposed the name *Neocosmospora vasinfecta*. Smith was able to obtain infection on the watermelon from cultures of a Fusarium isolated from the interior of wilted watermelon stems, but was unable to secure cross infections using the fungus isolated from the vascular systems of cotton, watermelon and cowpea, respectively. Likewise he was unable to secure infection of cotton plants by using the fungus isolated from the interior of cotton stems and secured only a few doubtful infections of cowpeas using the vascular Fusarium from cowpeas, which he was not able later to duplicate. In no case was Smith able to secure infection of cotton, cowpea or watermelon using the Pyrenomycete which he had isolated from the exterior of cotton stems.

Orton (1900) accepted the *Neocosmospora vasinfecta* of Smith as being the pathogene of cotton wilt. He found that the wilt usually begins to appear about the last of May when plants are 6 to 8 inches tall. Generally, at least 40 days after planting are required for the disease to become apparent. Well defined areas of diseased plants were found to appear, which increased from year to year. Orton considered that infection took place in the smaller roots independently of mechanical injuries or other fungi and noted the partial recovery of many plants. Repeated infection of smaller roots was noted especially in plants resistant to the disease without the fungus obtaining entrance to the tap-root. In such cases tufts of small roots appear at the points of injury and in many cases a considerable dwarfing of such plants was noted without any of the ordinary symptoms of cotton wilt. Soil inoculations using the conidial stages of Neocosmospora were successful, the incubation period being from 35 to 50 days. It is apparent that for these infections Orton employed cultures of the vascular parasite isolated from the interior of cotton stems. Orton finally records the failure of various soil fungicides and describes in considerable detail the first work on the selection of wilt resistant strains of cotton. In this work it was repeatedly noted that resistant plants were resistant because of the inability of the pathogene to enter the tap-root and that marked killing of small roots, accompanied by dwarfing, took place. In all such cases tufts of small roots replace those killed by the wilt fungus. It was finally noted that in some of the better resistant strains a lesser number of small roots were attacked than in others.

Fulton (1907) described the symptoms of cotton wilt and noted that it was widespread. The disease prefers a light, well drained, sandy soil, but it is often as severe in Louisiana on fer-

tile land as on exhausted soils. It spreads in the soil from year to year. The pathogene of cotton wilt was not isolated from seed and Fulton was unable to obtain any evidence that the disease is seed-borne. Although cotton wilt was noted to increase when land is cropped continuously to cotton, seven years in other crops did not completely eliminate the disease from the soil. Fulton noted the increase of the pathogene in the soil due to the presence of diseased stalks and the use of barnyard manure inoculated with the cotton wilt pathogene, but noted that "the condition of the soil has another indirect bearing on the wilt problem growing out of the important part the soil plays in the production of vigorous cotton plants that can ward off disease. Along this line it has been shown that the use of stable manure on wilt-infested lands results in a very marked decrease in the loss from wilt, as well as in the increase in the productiveness of the stalks generally." Twice as much loss was noted on unmanured plots as on plots manured at the rate of 30 loads per acre. Cowpeas and other legumes used as a green manure were noted to have similar though less marked effects while commercial fertilizers had no effect on the incidence of cotton wilt.

Orton (1907) noted that on Sea Island cotton, "Wilt is most prevalent on sandy or sandy loam soils best suited for Sea Island cotton" and that "Low spots and rich bottom lands often escape the disease long after the cotton on the higher portions of the field is killed". He noted the comparative absence of wilt in clay soils and emphasized the fact that wilt is due to a parasitic fungus rather than to any unfavorable soil conditions. According to Orton: "Wilt is not due to the continual use of commercial fertilizers, nor to the exhaustion of any element of plant food". He further noted that "the free use of stable manure will assist in the control of wilt by increasing the vigor of resistant varieties" but that "ordinary non-resistant kinds are not sufficiently protected by stable manure". Orton noted also the beneficial effect of plowing under leguminous crops and compost.

Orton (1908) noted the wide distribution of cotton wilt in the eastern cotton belt and in Louisiana and Mississippi, but recorded it only from one station in Arkansas. Wilt was found to be most prevalent in the sandy soils of alluvial origin and rare in the clayey soils of the Piedmont except in scattered areas of sandy and alluvial soil. According to Orton, wilt first appears in May or June when cotton is 8-12 inches high and is most prevalent during June and July, but continues to develop until the end of the season. The time of planting had little effect on the incidence of wilt and no benefit could be noted from late plantings, but on the contrary, early plantings gave as a rule the best crop. Orton again emphasized the relationship of light, sandy soils to the incidence of wilt and stated that the disease

is perhaps worse in soils lacking organic matter, although it may be very serious in rich and highly manured land. Efforts to remedy wilt by the use of green manures were found to generally result in an increase in the amount of the disease due to the use of the root-knot susceptible varieties of cowpeas for this purpose. According to Orton "Wilt is less injurious to cotton on low land or in low spots in a field. These are richer in organic matter and are also wetter. Probably both factors are involved". He noted that wilt had not been observed in black prairie soils, and stated that no benefit from the use of any type of commercial fertilizers had been found. Referring to the use of stable manure he says, "Our experience has been that in slightly infected fields this does give some relief, but the wilt takes the field in the end in spite of the heaviest manuring."

Higgins (1909) working in North Carolina on the wilt of cowpeas, was unable to prove the relationship of *Neocosmospora vasinfecta* (Atk.) Smith to *Fusarium vasinfectum* Atk. and presented very strong evidence to disprove any such connection.

Butler (1910) working in India with the wilt disease of chick-pea also concluded that *Neocosmospora vasinfecta* was merely a saprophyte appearing on the dead stems of various plants affected with Fusarium wilt.

Lewis (1911) found that no measure of control for cotton wilt was afforded by the application of fungicides to the soil even in large quantities. The disease was found to be much more severe on loose sandy soils, the worst affected spots being those low spots in the fields where sand is washed in, forming a loose sandy soil. Such a soil, when analyzed chemically was found to contain 90 per cent of silica, but was not deficient in plant foods with the exception of calcium. Experiments with liming to replace this deficiency had no effect whatsoever on the development of cotton wilt. Lewis also noted the absence of cotton wilt in the clayey soils of Georgia. Numerous fertilizer tests including plots treated with phosphate, muriate of potash, kainit, fresh lime, tobacco dust and guano in various combinations and in some cases singly gave no definite results. The mortality in unfertilized plots was from 75 to 92 per cent while in the fertilized plots from 72 to 90 per cent of the plants died. The date of planting was not found to have any effect whatsoever on the development of cotton wilt. Lewis made note of the well known effect of root-knot upon the prevalence and severity of cotton wilt, recommended rotations with resistant crops for the elimination of root-knot as one of the important measures for the control of cotton wilt and reported at length on his work with wilt-resistant varieties of cotton.

Lewis (1915) reported further on the development of wilt resistant varieties of cotton, and noted the failure of as long

periods as ten years in other crops in eliminating the cotton wilt fungus from the soil.

Gilbert (1917) presented little new material concerning the conditions favoring the development of cotton wilt or with respect to control measures. It was noted that sandy ridges, high sandy spots and places where sand has been washed into fields are most subject to cotton wilt, that wilt is most severe in wet seasons and that new cases are more numerous after rains. According to Gilbert, wet seasons favor rapid growth of the plant and the pathogene, thus increasing the incidence and severity of the disease. The wilt fungus is said to obtain entrance through the wounds caused by the entrance of nematodes into roots.

Edgerton and Moreland (1920) in connection with their studies of the relation of soil temperatures to the development of tomato wilt called attention to the increased severity of the Fusarium wilt of tomato in reinoculated, sterilized soils over unsterilized, inoculated soils. They found that tomato wilt develops better around the edges of wooden flats than at other points in the flat, and suggest better aeration there as being an important factor in increasing wilt development. They ascertained the growth curve of *Fusarium lycopersici* in pure culture and found that the optimum temperature for growth is around 29°C., that the maximum temperature for growth is slightly above 37.5°C. and that fairly good growth occurred as low as 14°C. The growth curve for *Fusarium vasinfectum* in pure culture was also studied and it was found that "The curve of growth of the cotton fungus was almost identical with the tomato wilt fungus." It was noted that for this work several strains of *F. vasinfectum* were used. The optimum temperature for the growth of *F. conglutinans* was found to be somewhat lower. The optimum temperature for the growth of *F. lycopersici* was found also to be the best temperature for the development of tomato wilt. These workers noted further that the warmer temperatures of summer favor infection of cotton with *Bacterium malvacearum*.

Elliott (1921) noted the serious losses from cotton wilt in Arkansas and most of the other cotton states. He found that the disease was worse on lighter soils of poor water-holding capacity and that it was especially serious in the eastern part of the state, although present over most of Arkansas. According to Elliott, wilt is worse in wet seasons because of the sappy growth of the host and favorable soil conditions for the development of the pathogene.

Ajrekar and Bal (1921), studying a Fusarium wilt of cotton in India considered the question of the cause of wilting, and while they were unable to find the pathogene in sufficient quantities in the vessels of the cotton stem to result in sufficient plugging to cause wilting, they were also unable to secure wilt-

ing using aqueous solutions of an alcoholic precipitate from the culture solution upon which the pathogene had been grown. Their efforts to produce the disease by artificial inoculation gave very variable results from which they conclude that the Indian Fusarium producing cotton wilt is a somewhat weak parasite.

Rast (1922) reported the highly beneficial effects of kainit in increasing the yield of cotton and in apparently controlling cotton wilt.

Elliott (1923) adduced evidence showing that cotton wilt is at times carried in the interior of the seed and that artificially inoculated seed carried the fungus in a viable form on the seed lint for at least five months. The disease was introduced into wilt-free soil by means of artificially infected seed.

Dastur (1924) attempted to infect cotton plants with strains of Fusarium isolated from wilted cotton plants in India, by scraping away the soil from around plants and placing the inoculum in contact with the lateral roots, which in some cases were injured and in other cases not. The soil was then replaced. No infections took place either in tests or in uninoculated controls. Dastur also failed to isolate a Fusarium or other pathogene from many wilting plants and concludes that the vascular Fusarium of wilting cotton plants is only incidental and secondary to some other type of injury. He attributed the wilting of cotton plants to the accumulation of iron and aluminum compounds in their tissues, which he identified by microscopical tests.

Kottur (1924) reported successful efforts to secure strains of cotton resistant to wilt in India, and noted the increasing importance of wilt in India and the deleterious influence of continuously cropping with cotton when wilt is present.

Rosen (1925) reported a type of damping off of cotton seedlings caused by *F. vasinfectum*, which is differentiated from the damping off caused by Rhizoctonia by a darker, blackish color in the case of the type caused by *Fusarium vasinfectum* and by the occasional presence of the pathogene of wilt in the vascular system of the young plants.

McRae (1926) studied the temperature relationships of the cotton fungus *Fusarium sp.*, using intervals of five degrees centigrade, at temperatures from 20°C. to 40°C. The optimum temperature for the growth of the pathogene on both a liquid medium and on an agar medium in petri dishes was found to be between 25°C. and 30°C.

Rosen (1926) carried out studies on the mechanism of wilting in cotton wilt. Using filtrates from various media including Richards' solution and Uschinsky's asparagin solution, upon which the cotton wilt pathogene had been grown, it was shown that media such as Richards' solution, containing inorganic nitrogen, upon which the pathogene has been grown, became

very toxic to cotton plants while media such as Uschinsky's asparagin medium and peptone broth do not. Rosen suggested that soils lacking in organic matter may be more favorable to the development of wilt because under these conditions the cotton wilt fungus may produce more toxic products than when more organic nitrogen is present. He further found that the filtrates from Richards' solution upon which the pathogene has been grown contain at least two toxic substances. "One is a volatile compound, slightly alkaline in reaction, and the other an inorganic salt, in the form of nitrite." "As the fungus is not present in sufficient quantity within the xylem to account for the wilting, and as wilting is at times obtainable in the absence of the fungus, it seems logical to conclude that the pathological phenomena here discussed are due primarily to certain poisonous chemical compounds produced by the fungus." "The wilting of cotton infected with *Fusarium vasinfectum* is considered to be due to poisonous chemical substances formed by the fungus."

Bal (1926) considered briefly the suggestion of Dastur (1924) that wilting of cotton in India is due to the accumulation of iron and aluminum salts in the plant. Bal found that the cotton soils of the Central Provinces of India showed P_H values varying from 8 to 8.8 and adduced evidence from numerous sources to the effect that aluminum salts do not remain in solution in alkaline soils. He, therefore, dismissed Dastur's hypothesis as untenable under conditions obtaining in the cotton soils of the Central Provinces. Dastur's findings that there is a greater accumulation of aluminum in wilted than in unwilted plants may, it is suggested, merely indicate a derangement of the absorptive processes of the roots due to the action on them of the pathogene.

Butler (1926) working in India carried out inoculation experiments using a Fusarium isolated from wilted cotton plants. Although the percentage of infection was low and the incidence of the disease irregular, infected plants showed characteristic symptoms of cotton wilt and Butler concluded that the experiments show "that the disease is caused by a Fusarium which is capable of pathogenic action under certain conditions not yet elucidated, and that these conditions are probably not connected with the composition of the soil, as has been suggested by J. F. Dastur."

Young (1926a) in a preliminary report on the relation of soil temperature to the development of cotton wilt found that the maximum temperature for wilt development was above 35°C., that the optimum was near 32.5°C. and that the minimum was near 25°C.

Young (1926b) reported that 30° to 32°C. appeared to be the optimum temperature for the development of cotton wilt. Wilt appeared at 35°C., the highest temperature then employed, and

for practical purposes 25 C. was considered near to the minimum.

Young (1926c) reported further that temperatures above 35 C. result in very slight amounts of wilt and that a temperature of from $37\frac{1}{2}$ C. to 40 C. was very detrimental to the cotton plant and resulted in a stunted type of growth. The importance of thorough mixture of cotton wilt inoculum into the soil was noted.

Young (1927) reported that temperatures from 26 to 32 C. are rather favorable to the development of wilt. The detrimental effects on the cotton plant of soil temperatures above 35 C. were again noted, and the rapid emergence of cotton at favorable warm temperatures as compared to slightly lower temperatures was recorded. The possibility of using actual soil temperatures as a criterion for planting cotton to avoid slow germination and its attendant evils of damping-off and seed decay was suggested.

Rosen (1927) called attention to his work reviewed above and suggested that in view of his finding that filtrates from cultures of the cotton wilt pathogene grown on media containing organic nitrogen were not toxic to cotton plants, while similar filtrates from media containing inorganic nitrogen produced substances toxic to cotton, the use of organic fertilizers is a promising method of controlling the disease. It was further suggested that the fungus growing in the soil may by its secretions injure the roots of cotton and that *F. vasinfectum* is a wound parasite and invades roots only after injury to the roots by micro-organisms, nemas, and chemicals. He suggested further that the fungus lives a semi-parasitic life in the water tubes of the plant.

Lucky (1927), Fahmy (1927a) and (1927b) have presented brief summaries of work given in much more detail by Fahmy (1927c) and for this reason no review of these notes is included.

Fahmy (1927c) reported on extensive studies of the Fusarium disease of cotton as it appears in Egypt. Cotton wilt is widespread in the Delta region and Sakel, the most important long staple variety employed, is extremely susceptible to the disease. Ashmouni and Zagora, short staple varieties grown in Upper Egypt, are immune. Wilt is absent from Upper Egypt although a localized discoloration of the xylem was noted from which a Fusarium has been isolated. Fahmy prefers to designate the Egyptian type of disease as the Fusarium disease of cotton since wilting is not always a prominent symptom. A yellow network or mosaic, starting at one corner of a leaf and extending to the whole leaf, is a prominent symptom and the leaf may or may not dry up. The mosaic symptom is more frequent in the seedling stage and need not necessarily be present. At times the stem may start dying at the upper end and may

finally die or new shoots may be given off at the base of the plant. These new shoots may in turn be attacked. Affected plants may recover, but are dwarfed, much branched and poor producers. The roots of diseased plants when cut show an olive green discoloration of the xylem in the form of irregular bundles or streaks and this discoloration may extend upward from the roots, even reaching the growing point of the stem and the boll-stems. Hyphae of the pathogene in the vessels may be abundant enough to plug them or may be sparse and may be creamy white or yellow. The walls of the invaded vessels are stained with a yellow substance which appears to fill the small vessels. In some cases the cavities of cells are filled with a dark gum-like substance and tyloses have been observed. No spores are produced in the stems and the pathogene is confined to the xylem during the life of the plant. On dead stems, microconidia, macroconidia and chlamydospores appear in the order named. No sclerotia are formed. The spores germinate at 25 C. in water, but in some cases the chlamydospores fail to germinate. The fungus grows better on glucose media than on sucrose media, and the presence of citric acid favors the formation of chlamydospores. A staling substance produced on modified Richards' solution was found to cause wilting of cotton plants. Using acid potato plugs, as a medium, the fungus grows best at 30 to 35 C. The incubation period of the Fusarium disease on Sakel cotton varied from 12 days in July when the average daily temperature was 26.9 C. to 58 days in February when the average daily temperature was 15.9 C. When sown in March the first symptoms appear in from 60 to 90 days. Sakel is rated as being 96 per cent susceptible as compared to Ashmouni and Zagora which are immune. Other Egyptian varieties tested vary from 6 per cent to 73 per cent susceptible. Infection is possible at any stage of development but is most fatal on young seedlings. In warm weather the pathogene causes a species of damping off similar to that caused by the soreshin fungus. The mode of infection is through the uninjured roots and positive results have been secured by the injection of spores of the pathogene into the wood of cotton stems. In susceptible varieties the fungus develops in the xylem in the normal fashion when inoculated hypodermically, but in immune varieties only a local discoloration is produced. No evidence of the transmission of the disease through seed was secured. The disease appears in patches which enlarge until all the land is affected. Around these patches, the plants may show internal discolorations, but no external symptoms. In some fields internal discoloration of roots may be present without external symptoms of the disease being present. The infective capacity of the fungus varies with the amount of inoculum in the soil. Fahmy reported that the Fusarium disease of cotton is most serious

on the heavier fertile soils of the Delta, and that the use of stable manure tends to increase the incidence of the disease. Crop rotation has been found to be effective in reducing the amount of wilt. The pathogene was found at depths of a meter or more where it had presumably grown in cotton roots. Plants are subjected to many separate infections in soils favorable to wilt infection, but if only the first 20 to 30 cms. of soil are inoculated plants are apt to escape infection by growing into clean soil. Many local infections are said to be arrested by the formation of gummy material about the point of infection.

Fahmy made a comparative study of the morphological and cultural characteristics of the pathogene of cotton wilt as it occurs in America, India and Egypt. Micro- and macroconidia of all three Fusaria are similar, but certain differences in the shape and formation of chlamydospores were noted. The Egyptian Fusarium was found to differ materially from both the American and Indian forms when grown on rice medium, producing a brilliant yellow and light cobalt violet. Cultural characteristics of the Indian and American Fusaria were found to be more nearly alike, but all three reacted differently to different types of cotton. The Egyptian Fusarium was found to be a virulent parasite of long staple Egyptian and Sea Island varieties; the Indian strain was pathogenic on Indian varieties of cotton only, while the American strain was a weak parasite on Egyptian varieties, non-parasitic on Indian varieties and parasitic on some American varieties. The Egyptian Fusarium is considered by Fahmy to be distinct from both the American and Indian forms and the name *F. vasinfectum* var. *egyptiacum* is suggested for it.

Soil sterilization with carbon bisulfide and bare fallowing have not proven efficient methods of controlling the Fusarium disease of cotton in Egypt, but selections made in 1923 from the Sakel variety yielded four resistant strains of high grade Sakel which were very resistant to the disease in fields where 96 per cent of ordinary Sakel had died.

Singh (1927) working in India, described four distinct strains of Fusarium isolated from wilted cotton plants received from parts of the Central Provinces and Berar (India) two of which were considered identical with certain strains of Ajrekar and Bal (1921). Seven Fusaria were isolated from samples of "wilted" and "non-wilted" soil and an eighth strain from wilted cotton plants from Nagpur. Using all of the Fusaria mentioned above, inoculation experiments were carried out over three growing seasons with only negative results, although six months after inoculation these Fusaria were re-isolated from the inoculated soils.

Neal (1927) has reported work on a number of phases of the cotton wilt problem. In contradiction to Elliott (1924) Neal

found that wilt is more prevalent in hot dry seasons than in wet seasons. Using both petri dish cultures and liquid media, Neal found that the optimum range for the growth of *Fusarium vasinfectum* in pure culture was from 28 to 30 C. and that the maximum temperature at which growth occurred was about 38 C. Slight growth still appeared at 6 C. Growth in pure culture was found to be good in the more acid conditions and best when started in media adjusted to P_H 3.5. All series which were initially acid were adjusted toward the alkaline range by the growth of the fungus while all series started in alkaline media became less alkaline with the exception of a few started on the extreme alkaline side which first became acid and then reversed and became distinctly alkaline. In most cases total acidity changes were correlated with hydrogen-ion changes. Experiments similar to those of Rosen (1926) confirmed the latter's conclusions with respect to the toxic properties of culture media upon which the cotton wilt pathogene has been grown. Neal was unable to substantiate the work of Dastur (1924) with respect to the accumulation of iron in cotton plants affected with wilt. Using sterilized and unsterilized soil from a field heavily infested with wilt, Neal secured wilting only in unsterilized soils and concluded that Dastur's failure to secure results was due to his method of inoculation. Neal found it difficult to inoculate cotton plants even after 90 days if the fungus is placed only around the larger roots rather than being thoroughly mixed into the soil. Cotton plants were also grown in Shive's solution containing aluminum chloride and aluminum sulphate. When these salts were present in strength amounting to $^{N}/_{250}$ toxicity was apparent. After plants had been grown in solutions containing aluminum salts for 29 days they were found to be no more susceptible to cotton wilt than the controls. Aluminum salts were not found to be toxic in concentrations less than 36 parts per million and according to Neal the soils of the Southern United States in all probability do not have aluminum salts in solution in greater concentration than this.

Neal grew cotton in washed sand with various nutrient solutions in which phosphorus, potassium and calcium were individually omitted. Increased amounts of phosphorus and potassium were also used in certain experiments, and the effect of increasing and decreasing the amount of nitrogen was also tried. Iron and aluminum salts were also added in some cases. No consistent effects on the incidence of cotton wilt could be noted except with those cultures having increased amounts of potassium, with those from which calcium was omitted and with those having a high iron content. Field experiments gave some indications that potash may control wilt.

Tests of the comparative wilt resistance and money value of the crop were made with a considerable number of wilt resis-

tant varieties and certain non-resistant varieties, and it is recommended that "preference be given to wilt-resistant cottons in tracts where soil is heavily infested with the fungus."

REVIEW OF LITERATURE RELATING TO THE EFFECTS OF ENVIRONMENT UPON COTTON AND CERTAIN COTTON DISEASES

So far as the writer has been able to ascertain the classical work of Balls (1905), (1906) and (1908) dealing with the relation of soil temperature and other environmental factors to the development of soreshin of cotton is not only the first study of the relationship of soil temperature to the development of disease in cotton, but is also the first work of importance dealing with relationship of soil temperature to the development of disease in any plant. Since the writer is interested in securing as complete a picture as possible of the effects of soil environment upon the incidence and destructiveness of diseases of the cotton plant, the work of Balls and a few others is reviewed at this point.

Balls reported on the damping off of cotton seedlings in Egypt, which he identified with the soreshin disease reported in the United States by Atkinson in 1895. Balls investigated the temperature, moisture and oxygen relationships of the host and pathogene and found that the development of the disease was closely correlated with soil temperature and an abundance of oxygen. The pathogene grew best at 23 C. and its growth was soon completely checked at 33 C. due, as the author believes, to a form of self poisoning conditioned by higher temperatures. The optimum temperature for the development of the cotton plant is stated by Balls to be from 30 to 35 C., and he found that if cotton seedlings exposed to infection by the soreshin fungus are kept at 30 to 35 C. attack is inhibited and "the cotton, being at its optimum growth temperature, forms cork rapidly and resists attack, when the temperature is afterwards lowered." At lower temperatures, cork formation is less rapid while the rapid growth of the fungus is favored, resulting in severe infections. At 23 C. or below, soreshin developed in its severest form. Due to the requirement of the pathogene that both moisture and air be freely accessible, only those parts of the plant near the surface of the soil are attacked, and if by cultural methods the zone of sufficient moisture and the zone of sufficient oxygen can be prevented from overlapping, the disease will fail to develop. This Balls was able to accomplish by allowing the soil to become compacted around the stems so that the soil was insufficiently aerated below the surface for the development of the pathogene, while immediately at the surface the moisture supply was insufficient for the develop-

ment of the disease. Large amounts of organic matter in the soil were found to favor the development of large amounts of inoculum, thus favoring the development of the disease.

Balls (1919) reported that long time exposures of cotton plants to temperatures below 30 C. produced no injurious effects, while exposures to temperatures above 35 C. were definitely harmful, the maximum temperature for growth falling at about 37.5° to 38°C. The best temperature for the growth of cotton was found to be near 32°C.

Richards (1923) studied the temperature relationships of *Corticium vagum (Rhizoctonia solani)* on the bean and pea. In the course of this work Richards carried out some experiments with cotton also, continuing unpublished work previously initiated by F. R. Jones. Richards found that "The cardinal temperature for the pathogenicity of *Corticium vagum* remained the same for the various hosts—potato, pea, bean and cotton. Lesions occurred on all these plants from 9 to 29 C., with a general optimum for tissue destruction near 18°C."

Briton-Jones (1925) studied the conditions favoring the development of soreshin of cotton in Egypt and decided that the inhibiting effect of high temperatures on the development of the disease was not due to "its direct inhibiting effect on the growth of the fungus, but indirectly because of its accelerating effect on the growth of the seedling, and drying up of the upper layer of soil, which has an inhibiting effect on the growth of the fungus." He especially called attention to cultural practices giving a well drained soil and dry surface conditions as being most important in the control of this disease.

Godfrey (1926) studied the relationship of soil temperature to the development of root-knot caused by *Heterodera radicicola*. Although cotton was not employed in these experiments several species of susceptible plants were used with similar results. The minimum soil temperature for host injury was found to be about 13 C. Between 16 C. and 19 C. the amount of infection increased rapidly, and it was determined that from 20 C. upward to a point where soil temperature became unfavorable for host growth heavy infection was secured. Infection in slight amounts occurred even at 38 C., a temperature unfavorable for the roots of the plants used.

Massey (1927) has studied the effect of soil temperature both under controlled conditions and in the field on the development of angular leaf spot of cotton caused by *Pseudomonas malvacearum* on cotton seedlings. At temperatures from 10 C. to 15 C. infection was found to be slow and might be missed entirely in lightly infected seed; at 15 C. to 20 C. infection was general, but might be light if other conditions are favorable for the growth of cotton seedlings. At temperatures from 21 C. to 26 C. infection was severe. Above 26 C. and up to 28 C. the

infection decreased in intensity and above 28 C. very little infection resulted. At 30 C. the cotton plant was generally immune to this disease. The regional distribution of angular leaf spot of cotton is considered to be conditioned by soil temperature phenomena.

Park (1927) working in Ceylon reported the extreme importance of *Rhizoctonia solani* as a cause of the damping off of seedlings of many varieties of plants there. He studied the relationship of soil moisture and the humidity of the air to the development of damping off of seedlings by *Rhizoctonia solani*. Park's first work was with seedlings of *Cajanus indicus*, but cotton was found to be more suitable for experimental purposes and was employed for most of the work. Apparently no consideration was given to the relation of soil and air temperature to the development of the disease. Parks found that "in the range of soil moisture necessary for the successful development of cotton seedlings, a variation of water-content of the soils has little or no effect on the extent of infection of the seedlings. On the other hand, variations of the humidity of the atmosphere above the soil have an effect, and results obtained indicate that infection increases with an increase of humidity."

Camp and Walker (1927) using "a soil temperature tank based on an internally balanced system" which they believe to be more economical that the single unit type, studied the relationship of soil temperature to the growth of cotton. They found that the optimum soil temperature for the germination of cotton seed is 33 to 34 C. and that no germination takes place at 40 C. No germination was obtained at 14 C. or below, but they believe that under more nearly sterile conditions germination at 14 C. or below might be obtained. Between 15 C. and 35 C. the rate of germination was found to agree well with Van't Hoff's rule. Other factors besides temperature were found to largely limit the growth of cotton seedlings between 20 C. and 35 C. and it was found that temperature was a limiting factor only above that range.

METHODS AND MATERIALS USED

As was previously stated, the purpose of the work presented in this paper has been to determine if possible, the effects of different soil temperatures on the incidence and development of cotton wilt. So far as was possible other factors such as soil composition, soil moisture, air temperature, amount and source of inoculum and varieties of cotton used have been kept uniform for any one experiment, although slight variations have been made in the general plan for different experiments. Air temperatures were regulated in the greenhouse by thermostatic control supplemented by hourly hand regulation of ventilators during the period of day-light and up to 10 P. M. By experience

it was found that thermostatic control alone gave reasonably uniform regulation of temperatures during the night. Full records of the greenhouse temperature during the period of the experiments was kept by means of a recording thermograph which was checked daily by means of a set of maximum and minimum thermometers. Although sudden sharp variations of air temperture cannot be avoided by such methods, especially on days when there is a frequent variation from cloudiness to bright sunlight, the duration of these periods of sharp variation has been reduced to the minimum by careful hand-regulation of ventilators. By such regulation it is not uncommon to have the maximum and minimum readings vary no more than 4 degrees Centigrade during an interval of several days and during most of the time it has been possible to regulate within 1.5 degrees Centigrade of the desired air temperature for the greater part of the time when experiments are conducted within the period from November 1st to March 1st. All air temperature data was taken in a shelter similar in construction to the standard shelter used by the U. S. Weather Bureau for its instruments.

Soil temperature was controlled by means of Wisconsin Soil Temperature Tanks (see Fig. 1) which have been described on numerous occasions by L. R. Jones and his associates (Jones 1922) (Jones, Johnson and Dickson 1926) and also by Leukel

Figure 1.—Wisconsin Soil Temperature Tanks in operation for the purpose of determining the effect of soil temperature upon the development of cotton wilt.

(1924) and they need not, therefore, be described here. For heating purposes, the writer first employed the luminous, partial vacuum heater-bulbs recommended by Leukel and others, but it was found that the 200 and 500 watt bulbs employed required a heavier load than could be carried by the relays employed without excessive arcing and oxidation of the relay points. This often resulted in the points sticking, and since the heating capacity of these heaters is sufficient to raise temperatures in the tanks very appreciably above those desired within a comparatively short time if sticking of relay points occurs, it was found better to employ small carbon lamps capable of heating the tanks only a few degrees above the temperature desired. By varying the size and number of lamps in the heater-cans for the various temperature tanks according to the temperature desired, no serious oxidation of the relay points occurred and excellent temperature regulation was secured. While temperatures in the tanks varied at times as much as one degree Centigrade above or below the desired temperature, ordinarily the relays were sufficiently sensitive so that the variation was not more than a half degree Centigrade from the temperature desired. As previously noted Camp and Walker (1927) have recently described a temperature tank based on an internally balanced system which they claim to be more economical than the single unit type. During the winter months at Fayetteville, Arkansas, it has been possible to secure temperatures as low as 16 C. with no other cooling system than the use of city water and no difficulty has been encountered in balancing the cooling effect of a small stream of cold water against the heating system regulated by a mercury thermostat, a telegraphic relay and a storage battery. If the writer understands the system employed by Camp and Walker, it has the disadvantage of being less flexible than the single unit system in which the temperature interval between the tanks can be quickly regulated, bringing all units to the same temperature or to any other temperature desired.

The soil used in all six experiments herein described was secured from one location at Fayetteville, Arkansas, and by mechanical analysis was classified as "loamy fine sand".[2] Prior to use all soil was screened and sterilized in an autoclave for 4 hours at 6 pounds pressure. Enough soil for an experiment was then dumped on a previously disinfected concrete floor and thoroughly mixed and samples taken for the determination of moisture content and water-holding capacity. Check cans were then prepared by weighing out equal amounts of soil into containers. The remainder of the soil was immediately inoculated by shoveling and reshoveling repeatedly the soil and inoculum.

[2]The writer desires to acknowledge the assistance of Prof. W. H. Sachs, formerly of the Department of Soils of the Arkansas Agricultural Experiment Station, who performed this analysis.

the latter consisting of pure cultures of the cotton wilt pathogene on sterilized chopped cotton stalks or sterilized wheat bran. As will be pointed out later, the importance of thoroughly and uniformly mixing liberal amounts of the inoculum into all the soil cannot be over-emphasized.

In order that comparable results might be obtained, every effort was made to maintain uniform soil moisture throughout a series. The containers used consisted of galvanized soil cans 8 inches in diameter and 15 inches in depth. In the bottom of each can a small inverted flower pot was placed, the hole in the base of which had been carefully enlarged to accommodate a glass tube having a diameter of $\frac{3}{4}$ inch. The end of this tube was broken irregularly so that although it rested on the base of the can, water might easily escape into the interior of the flower pot. This arrangement is very similar to that used by Riker (1926) in his work on the relationship of soil environment to the development of crown gall, and provides an excellent means of watering the lower soil levels as well as of securing the proper aeration of the soil. Soil cans were watered by weight to a definite percentage of the water holding capacity of the soil, the water-holding capacity of the soil having been previously determined by the standard brass cylinder method. The soil cans with their watering tubes and pots having been brought to the same weight by use of pebbles, equal amounts of moist soil were weighed into all of the soil cans of an experiment, and it was then comparatively simple, having computed the actual amount of oven-dried soil in each can and the water-holding capacity of the soil, to determine the total weight of can, soil, etc. necessary to water the soil up to a previously decided upon percentage of the water-holding capacity. In most of the work the soil moisture concentration maintained was either 50 per cent or 65 per cent of the water-holding capacity of the soil, although certain cans at each temperature have in some cases been watered to other percentages of the water-holding capacity. By constant weighing the amount of soil moisture was not allowed to vary more than a few per cent from the desired concentration and by watering alternately the surface and the bottom of the soil (in the latter case through the glass tube and flower pot previously described) a rather uniform soil moisture was maintained.

Different varieties of cotton were employed in different experiments, but in each case care was taken to maintain comparable series at each temperature employed. The varieties used were: Triumph 156, Acala, a strain of Cook originally obtained from the Alabama Experiment Station, and a commercial strain of Trice largely employed in Arkansas. The strain Triumph 156 which was used in the first experiment was thought to be extremely susceptible to cotton wilt infection, but in the opinion

of the writer should be classed as only moderately susceptible. The strain of Acala used was probably less susceptible to infection than Triumph 156, but could not be considered "wilt resistant" in the generally accepted sense of the term. The strain of Cook used was extremely resistant to infection both in greenhouse experiments and in the field, while the strain of commercial Trice employed has proved to be extremely susceptible to wilt, although having apparently a slight admixture of moderately susceptible individuals, and was used for most of the work. In some cases the seed was delinted in concentrated sulphuric acid and in other cases it was not and in the last two experiments the plants were transplanted from small pots of sterile soil after they had become pot-bound, but in all cases great care was taken to have all the components of any one series strictly comparable in every respect so that an accurate basis for the measurements of soil temperature effects was secured.

In the first two experiments, the soil was insulated as soon as the cotyledons were well opened, by placing mineral wool over the soil surface around each plant, but tests of water leached through the mineral wool employed revealed the fact that the hydrogen-ion concentration of the water in contact with the mineral wool employed was adjusted considerably to the alkaline range by soluble materials in the mineral wool and for this reason ground cork was substituted in later experiments. Since ground cork does not absorb water it has the added advantage of allowing surface watering without any of the water being retained by the insulating layer. Frequent tests revealed the fact that both mineral wool and ground cork are very efficient in preventing the loss of soil moisture and in insulating the soil against the effects of external temperature.

Inoculum was prepared by inoculating flasks of sterilized chopped cotton stems or wheat bran with isolations of *Fusarium vasinfectum* obtained from wilting cotton from several locations in Arkansas. Original isolations were prepared by plating out pieces of diseased cotton stems on acidulated potato dextrose agar after having first thoroughly disinfected them with 1 to 1000 mercuric chloride and then washing them in two washings of sterile water. From these isolations several monosporic strains were secured. The soil for each experiment was inoculated with a composite inoculum made by thoroughly mixing several bran or cotton stem cultures of these monosporic strains together.

Altogether, six experiments were carried out covering a period of three years. In the first four experiments the soil having been inoculated and placed in the cans, was allowed to incubate for about ten days at greenhouse temperatures. The cans were then placed in the tanks and sufficient time was allowed for the soil temperatures to become adjusted to the temperatures of the

soil tanks. They were then planted with an excess of seed and as soon as the plants were sufficiently emerged the insulating material, mineral wool or cork as previously described, was placed around the plants. In the last two experiments, an attempt was made to overcome the unevenness of the original stand both with respect to numbers and size resulting from planting cotton seed in soils at different temperatures, by first growing the cotton plants in the greenhouse in sterilized soil in $2\frac{1}{2}$ inch pots until they became pot-bound, and then transferring them with as little disturbance of the soil and roots as possible to the previously inoculated soil of the temperature tanks. This method insured an even stand of seedlings and in many respects was found to be a more satisfactory procedure than that previously described. It is recognized, of course, that the method used for the first four experiments more nearly approximates field conditions for cotton.

Plants were examined daily and as soon as definite symptoms of wilt appeared the aerial parts were removed and used for plating out the causal organisms. This method was employed for the first four experiments involving several hundred isolations of *Fusarium vasinfectum*. Since in no case where fresh stems showing browning of the vascular elements were used was there a failure to recover the pathogene it was not considered necessary in later work to make reisolations from all diseased plants and subsequently only doubtful specimens in which the browning of the xylem was obscure were plated. It may be noted at this point that the writer in 1923 isolated from wilted cotton from one location in Eastern Arkansas, a Fusarium producing a yellow pigment and suggesting very strongly Fahmy's *F. vasinfectum* var. *egyptiacum* and that on another occasion he repeatedly isolated a species of Verticillium from stems of wilting cotton growing in the greenhouse at Fayetteville on a heavy clay soil. Neither of these fungi were used for inoculation purposes and they have not been encountered in later isolations.

EXPERIMENTAL RESULTS

During the course of the work reported herein six separate tests involving the effect of soil temperature upon the development of cotton wilt have been made. The earlier experiments were run for as long as a hundred days, but later results showed that there was no advantage in running experiments longer than 60 days and furthermore that beyond that time the greatly increased size of the plants and the greater amounts of transpiring surface made weighing and watering operations difficult if injury to the plants and excessive drying of the soil were to be avoided. Experiments I and II comprising work carried out during the winter of 1925-1926 and during the fall of 1926 were

largely preliminary in nature and due to certain defects in the technique employed were only partially successful. Triumph 156, Acala and Cook cotton were employed in these experiments. Later experiments were conducted with commercial Trice cotton, a variety which both field and greenhouse experiments had shown to be highly susceptible to cotton wilt. Chopped cotton stems were used as a source of inoculum for Experiments I and II, but it was found that inoculated wheat bran could be more evenly distributed throughout the soil and for the later experiments it was used exclusively. Likewise in the first two experiments, the inoculum was only slightly mixed with the upper layers of soil. These experiments, while giving results which agreed in general with those obtained later, resulted in only small amounts of wilt (only 26 per cent at the most favorable soil temperatures after 80 days) and for this reason these results were discarded since it was felt that without a greater range in the amount of wilt developing at different soil temperatures, no valid conclusions could be drawn. Later results have shown the extreme importance of thoroughly mixing the inoculum into the soil, a result which ploughing and much longer periods for the incubation of the pathogene in the soil no doubt accomplish under natural conditions. In soils as lacking in organic material as that used for these experiments the natural spread of *Fusarium vasinfectum* through the soil is apparently quite slow and when only a small amount of inoculum is added to the upper layers of soil the roots of cotton appear to pass through the inoculated zone before infection takes place, later becoming sufficiently resistant to infection so that in a large number of cases they escape infection even after several months exposure at favorable soil temperatures. The work of Dastur (1924), Fahmy (1927) and Neal (1927) confirms this point and indicates further the necessity for thoroughly inoculating all the soil through which the roots spread if uniformly high percentages of infection are to be secured. The detailed results of the last two experiments together with a complete summary of the data from the last four experiments are presented below.

Results of Experiment V. Nov. 7, 1927-Jan. 5, 1928.—Seven temperature tanks were employed in this experiment, soil temperatures from 18 C. to 33 C. being employed with intervals of 2.5 C. The air temperature was maintained as nearly at 29.5 C. as possible. Certain series at each soil temperature were watered to different percentages of the water-holding capacity of the soil, care being taken to keep the soil uniformly moist by frequent weighings and by watering from the upper and lower surfaces as explained previously. Since no significant differences in the amount of cotton wilt appeared within the range of soil

moistures employed, the results of soil temperature work cannot be said to have been influenced by the differences in the soil moistures employed. Each tank contained eight soil cans, the depth of the soil being in each case about 12 inches. One can from each tank was reserved for a check and the number of plants in each can was approximately eight. As previously explained, the soil in the cans was thoroughly inoculated and watered to a good condition of tilth and the cans were then covered loosely and allowed to incubate for ten days at greenhouse temperature. Cotton plants of ordinary commercial Trice which had been grown in $2\frac{1}{2}$ inch pots until pot-bound and until the first true leaves were beginning to open, were then transplanted, with as little disturbance of the roots as possible, to the soil cans and the surface of the soil insulated to a depth of 2 inches with ground cork. The results of this experiment are extremely clear cut and although the temperatures employed do not extend throughout the complete range of cotton wilt infection, they indicate very clearly the optimum range and the close proximity of the maximum and minimum temperatures. Typical symptoms of cotton wilt appeared at temperatures from 28 C. to 33 C. by the twelfth day and during the next twelve days the greater part of the wilting at the more favorable temperatures occurred. The numerical results of this experiment are presented below in Table 1 and Fig. 2, the results being tabulated for intervals of 12 days from date of transplanting until the experiment was concluded at the end of 60 days. (See also Figures 3, 4 and 5.)

TABLE 1. THE RELATION OF SOIL TEMPERATURES FROM 18°C. TO 33°C. TO THE DEVELOPMENT OF WILT IN TRICE COTTON IN ARTIFICIALLY INOCULATED SOIL. (Experiment V.)

No. of plants in stand	Soil temperature °C.	Percentage of diseased plants at designated periods after planting in artificially inoculated soil				
		12 days	24 days	36 days	48 days	60 days
57	18	0	0	0	10.5	10.5
55	20.5	0	1.8	18.2	27.2	32.7
76	23	0	8.9	19.6	33.9	35.7
54	25.5	0	22.2	37	48.1	50
58	28	1.7	39.7	46.6	55.1	55.1
64	30.5	12.5	45.3	62.5	71.9	76.5
59	33	3.4	23.7	35.5	47.4	52.5

The optimum temperature for the development of cotton wilt is shown in Table 1 and Figure 2 to be between 28 C. and 33 C. and appears to fall rather close to 30.5 C. Since the interval between temperatures is 2.5 C., it is possible that the true

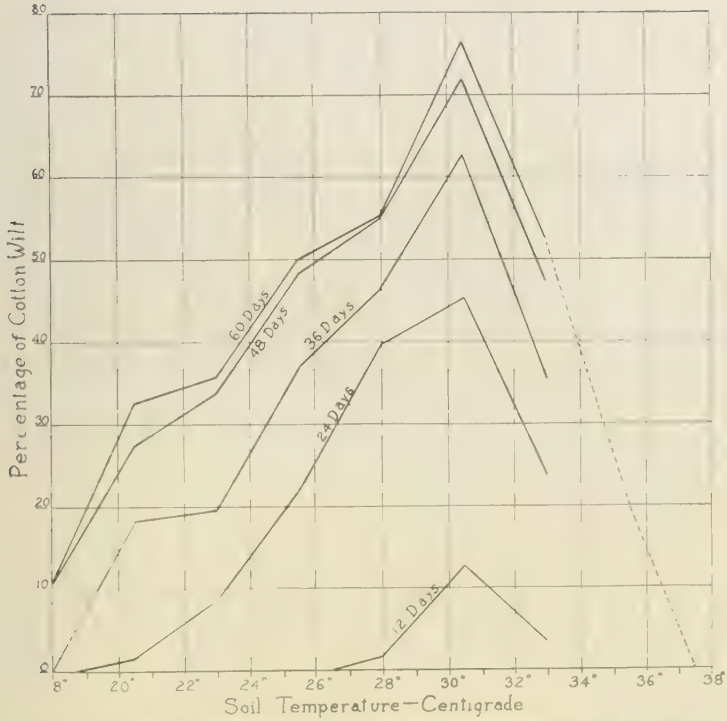

Figure 2.—The influence of soil temperature on the incubation period and development of cotton wilt at the end of 12 day intervals from the 12th to the 60th day. (Experiment V. Nov. 7, 1927-Jan. 5, 1928.) The dotted line indicates the probable result at higher temperatures as indicated by data from other experiments.

optimum may fall slightly above or below this point. The shortest period at which infection was noted was 12 days, but the fact that at the end of twelve days 12.5 per cent of wilt was found at the most favorable temperature employed indicates the probability that more careful scrutiny might have revealed a small amount of wilt even previous to this time. The curves shown above not only indicate the effect of favorable temperatures in shortening the incubation period, but indicate also that between the twelfth and twenty-fourth day the rate of wilting was much greater than for any other twelve day period, and that after the twenty-fourth day the actual rate of infection declines rapidly, apparently either showing a decreasing susceptibility on the part of the host with increasing age, or the early elimina-

tion of the more susceptible individuals of the population. The skewed curve having its highest point to the right of the middle point agrees rather closely with the curves given by other workers who have studied the relationship of soil temperature to the development of Fusarium wilts and also with the curves representing the relationship of temperature to many other life processes.

Figure 3.—The effect of soil temperature upon the development of cotton wilt at the end of 60 days. Temperatures left to right from 18° C. to 33°C. with intervals of 2.5°C. Upper row, sterilized soil uninoculated, no cotton wilt present. Lower row, sterilized soil inoculated with the cotton wilt pathogene, no wilt at 18°C. The greatest amount of wilting took place at 30.5°C. and somewhat less at 33°C.

Results of Experiment VI. Jan. 10 to Feb. 14, 1928.—This experiment was in every way similar to Experiment V, except that it was continued for only 36 days and that eight temperature tanks were used including soil temperatures from 18 C. to 35.5 C. The general technique of the experiment was not varied from that of Experiment V. Numerical results of Experiment VI which are similar to those of the previous experiment are presented below in tabular and graphic form. (Table 2, Fig. 6.)

Figure 4.—The effect of soil temperature on the development of cotton wilt. A and B inoculated soil held at 30.5°C.—all but two plants dead from cotton wilt. C uninoculated check held at 30.5°C.—no wilt. D inoculated and held at 18°C. E uninoculated check held at 18°C. No wilt in D or E

Figure 5.—The effect of soil temperature on the development of cotton wilt at 30.5°C. Six out of eight plants dead at the end of 36 days.

TABLE 2. THE EFFECT OF SOIL TEMPREATURES RANGING FROM 18°C. TO 35.5°C. ON THE DEVELOPMENT OF WILT IN TRICE COTTON IN ARTIFICIALLY INOCULATED SOIL.

No. of plants in stand	Soil temperature °C.	Percentage of diseased plants at designated periods after planting in artificially inoculated soil		
		12 days	24 days	36 days
62	18	0	0	3.2
62	20.5	0	8	14.5
58	23	0	27.5	39.7
65	25.5	0	33.8	43
61	28	4.9	42.6	47.3
67	30.5	4.5	46.2	62.7
58	33	8.6	46.5	55.2
58	35.5	3.4	15.5	20.7

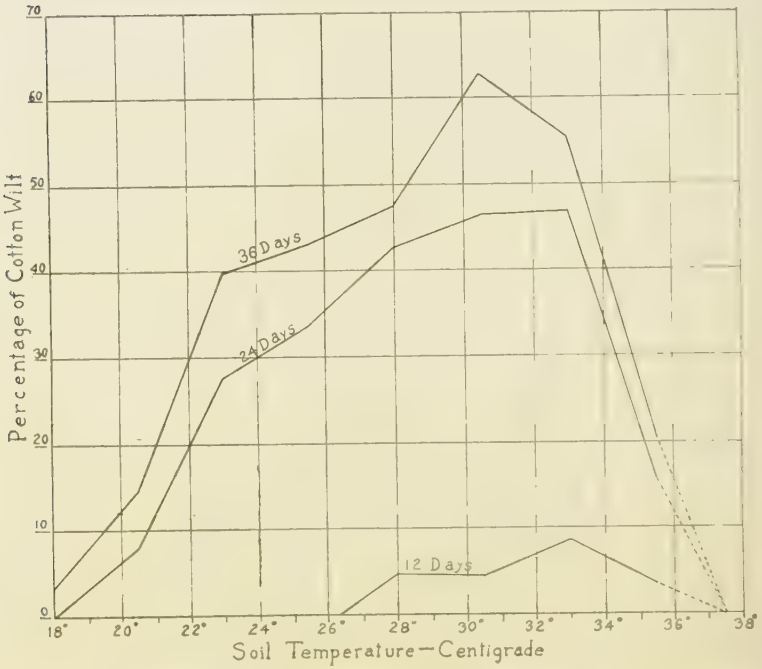

Figure 6.—The influence of soil temperature on the incubation period and development of cotton wilt at the end of 12, 24 and 36 day periods. (Experiment VI, Jan. 10, 1928, to Feb. 14, 1928.) Dotted line shows probable effect of higher temperatures as shown by other experiments.

Results shown in Table 2 and Figure 6, giving the results of Experiment VI, conform rather closely to those of Experiment V and in addition show the continued rapid decrease in the incidence of cotton wilt at higher soil temperatures. The results of three earlier experiments which indicate rather clearly that the maximum temperature for the development of cotton wilt is below 38° C., are shown by dotted lines and indicate the tendency of the curve to drop off still more sharply beyond 35.5° C. The shifting of the optimum to the right for the 12-day curve is probably without significance since the total amount of wilt at the end of 12 days at any temperature was extremely low.

For the sake of comparison the results of the first 36 days of Experiment V and the results for Experiment VI are shown combined in Table 3 and Figure 7, and Experiments III and V are shown graphically in Figure 8. The combined results of Experiments III to VI are shown graphically in Figure 9.

TABLE 3. THE EFFECT OF SOIL TEMPERATURES RANGING FROM 18°C. TO 35.5°C. ON THE DEVELOPMENT OF WILT IN TRICE COTTON IN ARTIFICIALLY INOCULATED SOIL.
(Combined results of Experiments V and VI.)

No. of plants in stand	Soil temperature °C.	Percentage of diseased plants at designated periods		
		12 days	24 days	36 days
119	18	0	0	1.7
117	20.5	0	5.1	16.2
114	23	0	18.4	29.8
119	25.5	0	28.6	40.3
119	28	3.4	41.2	45.4
131	30.5	8.4	45.8	62.6
117	33	6.0	35.0	45.3
18	35.5	3.4	15.5	20.7

As was previously noted, the results of Experiments I and II were discarded because of the low incidence of wilt in them, although the results agree in a general way with those of the succeeding four experiments. The results of Experiments III and IV agree closely with later results except that the total amount of wilt at all temperatures was somewhat less. This is to be explained by the fact that up to the initiation of Experiment V, greenhouse temperatures were maintained at 24° C. for the benefit of other work, a temperature which later results have shown is decidedly less favorable for the development of the pathogene and of vascular browning in the stems than was the temperature 29.5° C., later employed. Although examination

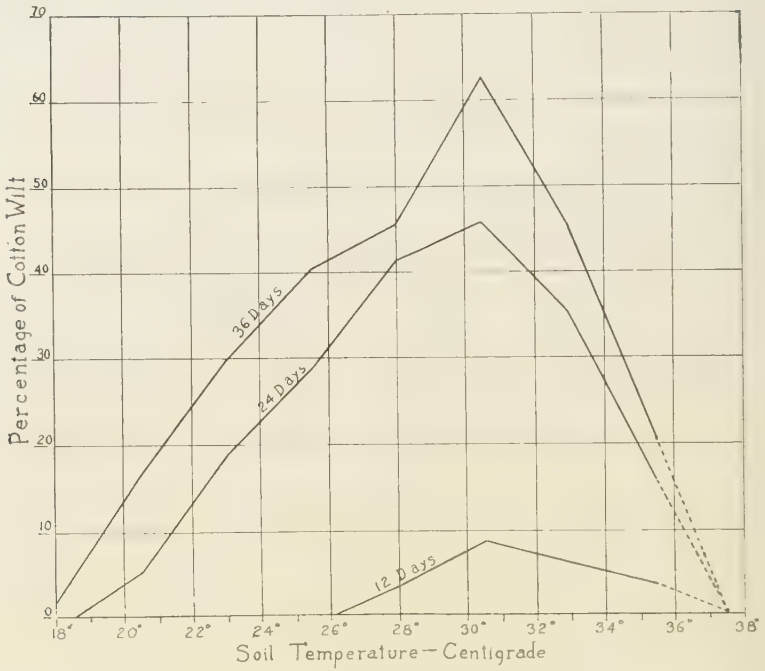

Figure 7.—The influence of soil temperature on the incubation period and development of cotton wilt, shown at the end of 12, 24 and 36 day periods. (Combined results of Experiments V and VI.) (Dotted lines show results for earlier work in which temperatures up to 40°C. were used. Results from 33°C. to 35.5°C. are for Experiment VI only.)

at the close of Experiments III and IV disclosed the fact that at favorable soil temperatures the roots of many plants showed vascular browning without browning or other visible symptoms in the aerial parts, such difficulty was encountered in securing pure cultures of the pathogene from many of the roots that these results were discarded. However, a comparison of the results secured when a lower air temperature was employed with those of later experiments in which the greenhouse temperature was maintained at about 29.5 C. reveals the fact that the percentage of plants at 24 C. showing wilting at a given soil temperature plus the percentage of plants at the same soil temperature showing the vascular darkening in the roots only is approximately equal to the total percentage of wilt resulting at the same soil temperature when the air temperature was maintained at 29.5 C. It thus appears that although air temperatures markedly affect the rate of migration of the cotton wilt pathogene into the stem

the invasion of the roots is primarily a matter of soil temperature and is little affected by the temperature of the air.

Correlation of Greenhouse work with Field Data.—The work of L. R. Jones and his associates in Wisconsin has shown clearly that the development of the Fusarium wilts of tomato, flax, cabbage and tobacco in the field is governed very largely by soil and air temperatures, and in many cases seasonal variations in soil and air temperature have been directly correlated with the incidence of the various Fusarium wilts. So far as the author knows, no soil temperature records in the field are available which can be directly correlated with cotton wilt development, but careful records of the development of cotton wilt in several varieties of cotton were kept during the season of 1926 at Galloway near Little Rock, Arkansas, and at the Cotton Branch Experiment Station at Marianna, Arkansas, during the season of 1927. Daily air temperature records are available for both of the stations, and while it is impossible to determine soil temperatures even approximately from air temperatures, an inspection of numerous records of soil and air temperatures for the same locality shown by Jones and his associates (1926)

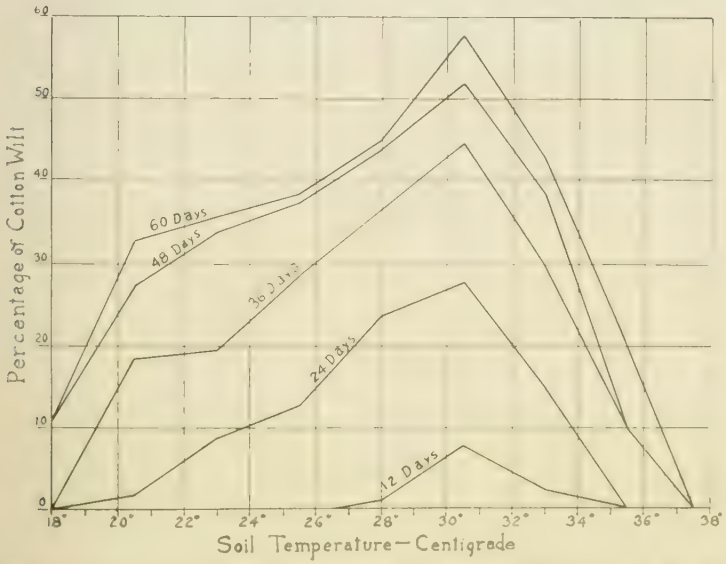

Figure 8.—The influence of soil temperature on the incubation period and development of cotton wilt shown at 12 day intervals from the 12th to the 60th day. (Combined results of Experiments III and V to 33°C. do not include results of Experiment V which included only temperatures from 18° to 33°C.)

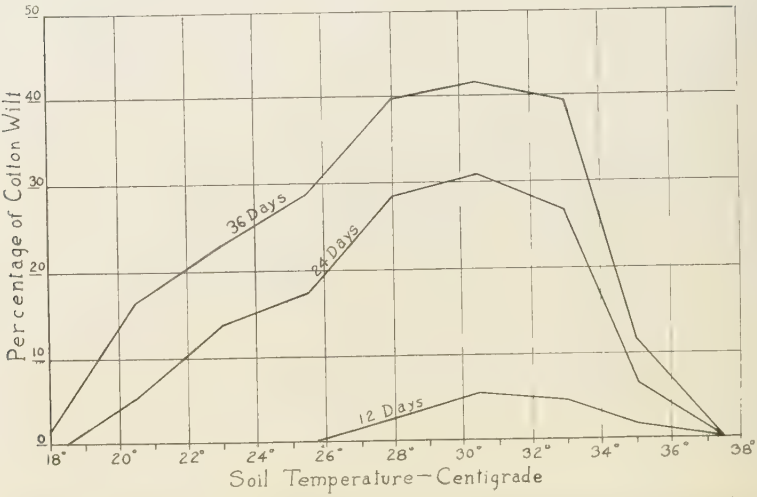

Figure 9.—The influence of soil temperature on the incubation period and development of cotton wilt as shown at 12 day intervals from the 12th to the 36th day. (Combined results of Experiments III, IV, V and VI.) Results at 35.5°C. do not include Experiment V, which included only temperatures up to 33°C. and results above 35.5°C. are for Experiments III and IV only.

show that soil and air temperatures tend to follow the same general trend with only a few degrees difference at most times. It is recognized, of course, that the data obtained at Galloway and Marianna, as mentioned above, allows the deduction of no definite conclusions. However, the results seem to show a certain amount of correlation between air temperatures and the development of cotton wilt, and are presented herewith in lieu of more satisfactory data.

TABLE 4. THE RELATION BETWEEN FIELD TEMPERATURES AND THE DEVELOPMENT OF COTTON WILT. GALLOWAY, ARKANSAS, 1926.

Variety	No. plants	Per cent wilt 33 days (June 6)	Per cent wilt 66 days (July 9)	Per cent wilt 95 days (Aug. 7)	Per cent wilt 150 days (Oct. 1)
Trice	159	1	33	87	87
Delfos	128	tr.	8.5	47.5	59
Webber	129	tr.	13	61	64
Rowden	124	tr.	9.5	46	52
Cook	137	0	0.7	3.5	9
Dixie Triumph	133	0	1.5	4	17
Super 7	100	0	1	6	26

Cotton planted May 4th. (Mean monthly temperatures during this period were as follows: May, 21.3°C.; June, 25.5°C.; July, 27.2°C.; August, 27.2°C.; September, 25.2°C.)

It will be seen from Table 4 that no wilt developed from May 4th, the date of planting, until the first counts were made 33 days later, the only definite cases being noted in the very susceptible Trice variety. Under greenhouse conditions at favorable temperatures a measurable amount of wilt appears in such varieties as Trice by the end of the 12th day, and by the end of the 24th day the rate of wilting has begun to subside. The results of the work in 1926 at Galloway show that wilting was only fairly under way at the end of 66 days and that the maximum amount of wilting took place from July 9th to August 7th or between the 66th and 95th days. Resistant varieties such as Cook, Dixie Triumph and Super Seven, which showed 9, 17 and 26 per cent of wilt respectively by the end of 150 days had only 7, 1.5 and 1 per cent of wilt respectively at the end of 66 days. As will be seen from the final results, conditions were probably as favorable for wilt as are commonly encountered anywhere in the United States. The soil was very sandy Arkansas River bottom land and a considerable amount of root-knot was present. Under these conditions, a killing of over fifty per cent in a semi-resistant variety such as Rowden and almost complete killing of Trice cotton resulted. One must conclude that field conditions during the first 33 days after this cotton was planted were extremely unfavorable for the development of cotton wilt and that during the next 33 days (the rest of June and early July) conditions must have not been at all at the optimum. During the next 29 days (the last 22 days of July and the first seven of August) the greatest incidence of wilt occurred and during the rest of the season very little wilting took place except in the three very resistant varieties in which wilting apparently did not get well under way until August.

Air temperatures during May (mean temperature 21.3 C.) and probably during early June were decidedly lower than have been shown in temperature tank tests to be favorable to the development of cotton wilt, whereas the mean air temperature

TABLE 5. THE RELATION BETWEEN FIELD TEMPERATURES AND THE DEVELOPMENT OF COTTON WILT AT MARIANNA, ARKANSAS, 1927.

Variety	No. plants	Per cent wilt 54 days (July 12)	Per cent wilt 83 days (Aug. 10)	Per cent wilt 111 days (Sept. 7)
Trice 304 (Plot A)	333	16.8	45.2	64.6
Trice 304 (Plot B)	333	14.7	49.6	66.8
Trice 304 (Plot C)	528	5.7	34.9	54.9
Express 116	456	1.8	7.9	24.6
Super 7	490	0	0	1.6

Cotton planted May 4th. Mean monthly temperatures during the growing period were: May, 23 C.; June, 25.6 C.; July, 27 C.; August, 24.7 C.; September, 24.6 C.)

for July and August fell very near to the optimum range. It seems apparent then that soil and air temperature must have been the principal factor in delaying the development of wilt in the field.

Results obtained at Marianna in 1927 in artificially inoculated soil gave less striking results so far as the total amount of wilt is concerned, but are nevertheless just as convincing as the data secured in 1926.

Except for the month of August, temperatures during the spring and summer of 1927 showed a positive departure. Cotton was not planted until well after the middle of May. The soil was artificially inoculated and was somewhat less sandy than that employed at Galloway. No root-knot infections were found. Conditions were apparently somewhat less favorable for the development of wilt, and the Trice 304 cotton employed was slightly less susceptible to wilt that the commercial Trice previously used. However, as in the previous year, more wilt developed in susceptible varieties during the last two thirds of July and early August than at any other time, whereas in more resistant varieties such as Express 116 and Super Seven the period of incubation was much longer and the maximum amount of wilt developed toward the end of the season. It seems likely that the unusually cool weather of August (showing a negative departure of 1.8 C.) may in part at least account for the smaller amount of cotton wilt developing in the 1927 work.

DISCUSSION OF RESULTS

In every important respect the relationship of soil and air temperature to the development of cotton wilt seems to agree very closely with the findings with respect to other diseases caused by vascular Fusaria. The optimum soil temperature for the development of cotton wilt in the six experiments reported upon has in every case been very close to 30.5°C., with a comparatively favorable range from 28 C. to 32 C. Above 33 C. the incidence of cotton wilt drops off rapidly and in no case has cotton wilt developed at 38°C., or above. In an earlier experiment carried out at 37.5°C. for a hundred days, one wilted plant developed from a stand of 40 plants at the end of 80 days. It seems reasonably certain, therefore, that no cotton wilt will develop at 38 C. and all experiments indicate that the incidence of cotton wilt at 35.5 C. will be extremely low. No temperatures lower than 18°C. have been employed in these experiments. At this temperature and using Trice cotton, a very susceptible variety, less than 4 per cent of all plants carried at this temperature were wilted at the end of 36 days, and at the end of 60 days only 10.5 per cent of wilted plants resulted. Further work using temperatures lower than 18 C. is necessary to determine the minimum temperature for the development of cotton wilt, al-

though for all practical purposes it seems unlikely that any appreciable amount of wilt will develop at temperatures below 23° C. where any except the most susceptible varieties are used. The curves and tables showing the relation of soil temperature to the development of cotton wilt are also closely correlated with the data secured by Edgerton and Moreland (1920) and Neal (1927) concerning the effect of temperature upon the growth of *Fusarium vasinfectum* in pure culture—a relationship which has been repeatedly noted by other workers studying other Fusarium wilts.

During the past twelve years the influence of soil conditions and more especially of soil temperatures on the development of the wilts caused by various species of Fusaria have been studied by numerous workers both under controlled conditions in soil temperature tanks in the greenhouse and by means of soil and air thermograph records in the field. In practically all cases, these studies have been coordinated with studies of the influence of environment on the growth of the pathogene in pure culture. In certain cases the relationship of the hydrogen-ion concentration of the soil and the influence of soil moisture have been studied. Much of this work has been carried on by L. R. Jones and his students at Wisconsin, although the excellent work of Edgerton and Moreland (1920) on the Fusarium wilt of the tomato in Louisiana should be noted in this connection. More recently Harter and Whitney (1926) and others have studied the relationship of soil environment to the Fusarium wilts. The outstanding result of all this work is the extreme importance of the effect of soil and air temperatures on the development of the Fusarium wilts. In most cases results have also shown a very close agreement between the optimal temperature for the development of wilt and the optimal temperature for the growth of the pathogene in pure culture, and in general the temperature growth curve of the pathogene and the curve showing the relation of temperature and more especially soil temperature to the development of the disease have been found to coincide to a remarkable degree throughout their length. In no case, so far as the writer is aware, has the optimum temperature for the development of a Fusarium wilt been either above or below a comparatively favorable temperature for the development of the host, although in cases such as that of the wilt of flax the two optima do not at all coincide.

Balls (1919) working in Egypt, stated that temperatures from 30° to 35° C. are most favorable to the development of cotton and Camp and Walker (1927) working in Florida found that the optimum soil temperature for the germination of cotton seed is 33° to 34° C. and that no germination takes place at 40° nor below 14° C. Other factors than soil temperature were found to limit the growth of cotton seedlings between 20° to 35° C. The

writer (1927) has reported briefly his observations on the effects of soil temperature on the germination and growth of cotton seedlings (Figure 10). Germination was secured at a temperature very close to 40 C., although it is probable that since no insulating layer was used at this temperature until the seedlings had emerged, the actual temperature of the first inch of soil may have been slightly below 40 C. Such plants, however, grew very slowly at 40 C. and even after 100 days were barely beyond the seedling stage and in a very unthrifty condition. Soil temperatures above 35.5 C. are unfavorable to the growth of cotton and check its growth. Within the soil temperature range from 20.5°C. to 35.5°C. cotton plants grew fairly well. However, there was some checking of growth below 23 to 25 C. Plants grown at a soil temperature of 18 C. were decidedly stunted in their growth. It is to be noted further that below 25.5 C. cotton seed germination is increasingly less vigorous and at 23 C. and below the stand was often seriously diminished due to decay of seed and damping off of seedlings. Cotton seed which had been delinted with sulphuric acid germinated and the plants emerged much more rapidly than when seed was not delinted, the first plants appearing at 35.5 C. in two days, whereas with undelinted seed germination was delayed fully two days. From what has been said, it is apparent that cotton can probably survive in soils having temperatures of 38 to 40 C., whereas no cotton wilt can develop at these temperatures and very little for at least one or two degrees below 38 C. Although no definite data are available with respect to the relation of the minimum temperature for the development of cotton wilt to the minimum soil temper-

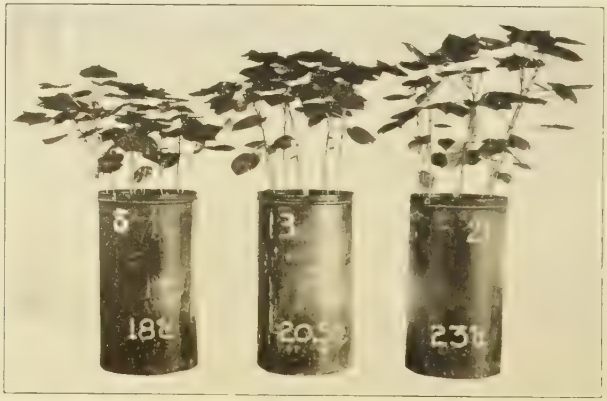

Figure 10.—The inhibiting effect of low soil temperatures upon the growth of cotton when air temperature, soil moisture and the nature of the soil are uniform. Left 18°C., center 20.5°C. and right 23°C.

ature at which cotton will germinate and grow, it seems likely from the trend of the curve for the incidence of cotton wilt that the cotton plant can survive at temperatures slightly below those at which cotton wilt will develop. It is apparent however, that the temperature range most favorable to the development of cotton wilt is well within the range of temperatures favorable to the growth of the cotton plant.

In this connection it is interesting to note that Clayton (1923) found that only vigorously growing tomato plants will develop tomato wilt in its characteristic form. The author has frequently noted that under both field and greenhouse conditions those plants which are delayed in germination and remain in a more juvenile, spindling condition, due to crowding and shading of other plants, seldom or never develop the typical symptoms of wilt.

The earlier workers, Smith (1899), Orton (1900) and others, reported a comparatively long period of incubation for the development of cotton wilt, and the idea is prevalent that cotton does not become susceptible to cotton wilt infection until shortly before the first blooms appear. This is quite commonly the case under field conditions in Arkansas and, no doubt holds true for other cotton growing sections. Soil temperature studies in the greenhouse make it apparent that cotton seedlings are very susceptible to wilt infection and that with susceptible varieties and a favorable soil temperature, appreciable amounts of cotton wilt may develop within 12 days from the date of planting, and that the maximum rate of wilting may occur by the end of the 24th day. The incubation period apparently is very much prolonged in the more resistant varieties of cotton. As previously noted, Atkinson (1892) reported symptoms of cotton wilt in seedlings in the cotyledon stage and Fahmy (1927c) similarly reported that under field conditions the Egyptian type of cotton wilt may appear in 12 to 14 days during April to August when soil temperatures varied from 23.7 C. to 26.9 C., while the incubation period was prolonged to 58 days during the winter months when the soil temperature was 15.9 C. Neal (1927) has reported an incubation period of one week with cotton plants growing in a nutrient solution. These results make it clear that cotton seedlings are relatively susceptible to cotton wilt infection provided soil temperatures are favorable. Under field conditions, however, it is ordinarily 30 to 60 days after planting before soil temperatures become favorable for the development of cotton wilt. At present the value of early planting to escape boll weevil injury is well recognized. It now becomes apparent that providing good stands and rapid growth could be secured early planting might also be highly advantageous from the point of escaping early wilt infection. The selection of cotton for its

ability to germinate and grow at low temperatures as well as for its resistance to cotton wilt may therefore be advisable.

Fahmy (1927c) states that "infection is possible at any time during the life of a susceptible cotton plant," but that "the seedling stage is, however, the period when injury is most likely to be fatal". This statement implies at least that cotton seedlings are more susceptible to infection than are older plants. The writer suggests that from his experience with the soil temperature tanks it seems likely that the ordinary commercial strains of cotton are heterozygous with respect to wilt resistance. Any population of cotton plants, therefore, which has not been especially selected for a considerable period for wilt resistance, is composed of a relatively large number of susceptible individuals and a smaller number of individuals possessing more or less resistance. Under these conditions the more susceptible individuals when exposed to cotton wilt infection under optimum soil conditions wilt rapidly during the first twenty-four days, and from then on the more resistant individuals become infected much more slowly and behave very much like the greater per cent of the plants of a well developed wilt resistant variety.

Orton (1900) has noted the stunting effect of the cotton wilt pathogene on resistant varieties due to the killing of large numbers of secondary roots, even when the pathogene was unable to enter the tap-root. In this connection it is interesting to note that the few surviving individuals in a soil temperature tank after the majority of the plants have died from wilt often appear especially vigorous and due to the lack of competition from other plants, completely outstrip the plants in the uninoculated checks held at the same soil temperature. Such individuals appear to be comparatively resistant and thrive remarkably well even in highly inoculated soil.

The relationship of temperature to the growth of the cotton wilt pathogene is of considerable interest since it is desirable to determine the correlation between the effect of temperature upon the course of the disease and the effect of temperature upon the growth of the pathogene in pure culture. Edgerton and Moreland (1920) determined the effect of temperature upon the growth of several strains of *F. vasinfectum* from cotton and found that the curve of growth of the cotton fungus was almost identical with that of the tomato wilt fungus. Their results indicate that the optimum for growth of the two fungi was around 29 C., that the maximum was slightly above 37.5 C. and that fairly good growth occurred as low as 14 C. McRae (1926) reported that the optimum temperature for the Indian cotton wilt pathogene was between 25 and 30 C. Neal (1927) reported that the optimum temperature for the growth of *F. vasinfectum* in petri dish cultures was 28 C. while the greatest dry weight of the fungus in liquid media was at 30 C. According to Neal, the fungus

grows very slowly at temperatures below 10 C. while the maximum temperature for growth was found to be 38 C. The writer has determined the optimum for the growth of *F. vasinfectum* in petri dishes to be very close to 30 C., but has made no observations on the effect of temperatures below 25 C. or above 35 C. In general it will be seen that the curve of growth for *F. vasinfectum* in culture at different temperatures agrees very closely indeed with the curve representing the relation of soil temperature to the growth of cotton wilt, the optimum range for the development of cotton wilt and for the greatest development of the pathogene being almost identical. The trend of the two curves at the lower level is so similar as to lead one to suspect that the minimum for the growth of the cotton wilt fungus and for the development of cotton wilt are not far apart. It is interesting to note that the optimum temperature for the development of cotton wilt is apparently even higher than that for tomato wilt as determined by Clayton (1923), Edgerton and Moreland (1920) and Bewley (1922), who determined this temperature to be from 28 C. to 29 C. It is also worthy of note that what few field observations are available indicate that the minimum for the development of cotton wilt as an aggressive disease is if anything, also slightly higher than that for tomato wilt which is placed at 20 C. The maximum temperature for the two diseases is not far from being identical. Johnson (1921) has found that the optimum soil temperature for the Fusarium wilt of tobacco is from 28° to 31 C. and Harter and Whitney (1926) have determined that the soil temperature relationship of the stem-rot organisms (*F. batatatis* and *F. hyperoxysporum*) is much the same, although the maximum and minimum temperatures for the development of sweet potato stem-rot are somewhat higher and lower respectively than for the development of cotton wilt. It thus appears that the Fusarium wilts of tomato, tobacco, sweet-potato and cotton, four crops of tropical or semi-tropical origin, are favored by slightly higher temperatures than are the Fusarium wilt of crops of typically cooler regions such as flax and cabbage, which have been found to develop best at 26° to 28°C. and 26°C. respectively.

Atkinson (1892) and nearly all workers with cotton wilt since his time have noted that cotton wilt is of very slight importance in clayey soils, but is especially destructive in sandy soil and other soils which are inclined to be loose and friable. Fulton (1907) has added to this idea the fact that the soils favorable to wilt are also well-drained and may or may not be fertile. Orton (1907) noted further that low spots and rich bottom lands escape the disease as well as clay lands. Orton (1908) notes the relative absence of cotton wilt from the clay area of the Piedmont except in scattered sandy or alluvial spots. Lewis (1911) again notes that cotton wilt is most severe in the sandiest

parts of fields. Fahmy (1927) on the other hand, reports that the Egyptian type of cotton wilt is by far most serious in the heavier fertile soils of the Delta, but one must remember that Fahmy's description of the Egyptian type of wilt and of the morphology and physiology of the pathogene and its behavior toward American varieties of cotton make it very certain that he is dealing with a different disease than cotton wilt as encountered in the United States. Furthermore, it is not impossible that the physical properties of the Nile Delta soils may more nearly resemble those of our sandy alluvial soils than they do the clay soils which are least favorable in America for the development of cotton wilt. In Arkansas, the writer has had frequent occasion to note that wilt is almost certain to be much more severe in those sandy spots in fields commonly known as "sand blows" than elsewhere.

As has been previously noted, much of the data concerning the relationship of soil fertility, the use of stable manures and green manures and the use of such substances as nitrates, phosphates, ammonium salts, kainit and the like, separately and in combination, to the development of cotton wilt is so highly contradictory that the matter may be said to be in a completely unsettled state and in need of much fuller investigation under carefully controlled conditions of temperature, soil moisture, soil reaction and soil aeration.

Orton (1908) noted that wetter spots in fields are less subject to cotton wilt and Gilbert (1914) noted that wet seasons favor wilt by favoring the development of the pathogene and of the host. Elliott (1921) also noted that the greater incidence of wilt is in wet seasons, while Neal (1927) has noted and quotes Beal (1926) as confirming his observation, that wilt is worse in hot dry seasons. Clayton (1923), Tisdale (1923), and Harter and Whitney (1926) have noted that soil moistures except under very extreme conditions have little effect on the Fusarium wilt diseases. Unpublished work from this laboratory,[3] indicates that soil moisture at least when kept constant and reasonably favorable to the growth of the host does not appreciably affect the amount of cotton wilt developed.

Root-knot or nematode disease caused by *Caconema (Heterodera) radicicola* has been quite commonly noted to greatly increase the severity and amount of cotton wilt when present and according to Fulton the nematodes produce wounds through which the wilt pathogene may enter. However, there can be no doubt that cotton may be severely attacked in the absence of nematode infection. Fahmy has confirmed this point for the Egyptian type of wilt and the writer has repeatedly secured from 70 to 80 and even 100 per cent of wilt in sterilized soil

[3]J. R. Large, a graduate student, has been carrying out studies on this problem.

entirely free from nematodes within periods considerably shorter than the normal growing season of cotton.

Atkinson (1892) suggested the importance of wounding and the injury caused by the soreshin fungus as a point of entrance for *F. vasinfectum*, but Orton (1900) considered mechanical injury by other fungi as entirely unnecessary for the entrance of the cotton wilt pathogene and noted the repeated infection of smaller uninjured roots, even of resistant varieties. Dastur (1924) however, was unable to secure infection in India, even when inoculum was placed directly in contact with freshly cut roots. It is clearly still a matter of doubt whether or not wounding or other types of root injury, except root-knot are of any importance in affecting the development of cotton wilt.

Although Edgerton and Moreland (1920), Sherwood (1923) and others have noted some effects from liming soils on the development of tomato wilt, these effects have not been marked and no one has yet shown any clear relationship between soil acidity or alkalinity and the development of cotton wilt.

Rosen (1926) has suggested that the development of nitrites and other toxic principles in the soil and in the host through the agency of the pathogene is responsible for wilting of cotton, and he and others have shown conclusively the injurious effects of various substances produced in artificial culture solutions upon which various species of Fusaria have been grown upon the host when cut stems or even roots are immersed in the solution, but it seems to the writer that more work is needed before these conclusions may be accepted finally as demonstrating that toxic principles produced by vascular Fusaria are the only factors concerned in producing the wilting which is one of the principal symptoms of the diseases caused by vascular Fusaria. The observation by Clayton (1923) with tomato wilt and by the writer with cotton wilt that the pathogene may enter the main roots of the host and develop there for considerable periods causing distinct darkening of the vascular tissues of the roots without entering the stem when air temperatures are unfavorable and without producing vascular discolorations, wilting, yellowing or other visible evidences of wilt in the aerial parts, raises the question very definitely as to why under these conditions, the toxic principles produced by the pathogene in the roots do not pass along with the transpiration stream into the leaves and produce the wilting symptoms in the same manner that they pass into the cut ends of plants immersed in media upon which the pathogenes have been grown. In this connection, the finding by Fahmy (1927c) that the walls of the xylem elements of wilted cotton plants may be stained deeper yellow, that the same yellowish coloring matter may also fill some of the smaller cells near the invaded xylem cells, that other cells may be filled with a very dark gum-like substance and that

tyloses may also be formed is deserving of further investigation in connection with the problem of the cause of wilting in the vascular diseases caused by various species of Fusarium.

The work of the writer has shown a very definite relationship of soil and air temperature to the development of cotton wilt. No one doubts the serious effects of root-knot infection. The relation of soil moisture is somewhat obscure, but under favorable conditions for the growth of cotton at least probably exerts little influence. The chemical influence of the soil, i. e., the influence of various nutrient salts and of organic matter in the soil, remains yet to be fully elucidated. Clayey soils and very wet soils appear not to favor the development of wilt. Edgerton and Moreland (1920) suggest the possibility of greater aeration in the soil around the edge of wooden flats as an important factor in favoring the development of increased amounts of tomato wilt. The question naturally arises: Since the development of cotton wilt is clearly favored by sandy, well drained soils and inhibited by clayey soils or poorly drained heavy soils, may not the fact that sandy, well-drained soils are much better aerated and consequently favor the growth of the pathogene to greater depths so that it is in contact with more of the root system of the cotton plant, explain in part at least why such soils are more subject to cotton wilt? *Fusarium vasinfectum* in its manner of growth both in solid and liquid media does not appear at all anaerobic, in fact quite the contrary is the case. Clayey soils are naturally more poorly aerated and also offer much greater physical barriers to the growth of the pathogene through the soil. Likewise such soils hold the roots of the host much more firmly and there is less likelihood of mechanical injury in them. Every study of the relationship of soil factors to the development of the Fusarium wilts has pointed clearly to the fact that wilt is worse under those conditions which result in the greatest amount of infective material and in the greatest vigor of growth of the pathogene. The use of large amounts of inoculum and the thorough mixing of that inoculum throughout the soil has been commonly shown to greatly increase the incidence of wilt, and Neal (1927), Fahmy (1927c) and the author have shown that inoculum placed near the surface is much less effective than when mixed deeply into the soil. It may also be possible that sandy soils are consistantly warmer than clayey soils, but that point is in need of investigation.

All of these facts mentioned above point clearly to the great importance of large amounts of vigorously growing inoculum in the soil as one of the principal factors determining the incidence of cotton wilt. The conditions favorable for bringing about such a condition in nature appear to be a warm, well aerated, unsaturated soil containing sufficient amounts of organic matter to support a considerable saprophytic growth of the pathogene.

The modifying effects of wounding, of the chemical nature of the soil, of the soil reaction, and of pronounced variations in soil moisture on the host and parasite and on the incidence of cotton wilt and the effect of various manurial treatments on its development are in need of further investigation. Likewise, the effects of environment upon the wilt-resistant properties of the host should be more thoroughly studied.

Comparison of Cotton Wilt with Other Soil Borne Diseases of Cotton.—In view of the close relationship between root-knot infection and the amount and severity of cotton wilt, it is interesting to note that Godfrey (1926) who studied the relationship of soil temperature to root-knot infection found that in all cases studied vigorous infection may take place over a wide range, including all of the temperatures favorable to most crop plants. It, therefore, seems certain that the same condition is true with respect to the cotton plant, and that vigorous development of root-knot in cotton may not only take place throughout the range of serious cotton wilt infection, but also at much lower temperatures. Indeed it seems likely that when soil is infested with the root-knot nematode, cotton seedlings become infected almost immediately and are, therefore, in a highly susceptible condition as soon as favorable soil temperatures for the development of cotton wilt appear.

Balls (1905, 1906, 1908), Richards (1923), and Park (1927) have shown that soil temperatures below 25 C., moist soil and humid atmosphere at the surface of the soil favor the development of damping off and seed decay caused by the soreshin fungus (*Rhizoctonia solani*). Massey (1927) has recently shown that initial cotyledonary infection with the angular leaf spot pathogene (*Ps. malvacearum*) is closely coordinated with soil temperature. According to Massey temperatures from 21 C. to 26 C. favor severe infection while at lower temperatures less infection takes place although infection may take place even at 10 C. At temperatures above 28 C. very little infection takes place and at 30 C. the cotton plant is generally immune to this disease. It thus appears that both soreshin and initial angular leaf spot infection are favored by soil temperatures distinctly below those favorable to cotton wilt infection, whereas at the temperatures most favorable to the development of cotton wilt neither angular leaf spot infection in the soil nor soreshin is of any importance whatsoever.

SUMMARY

1. Cotton wilt, the most important disease of cotton in the United States is especially prevalent in well-drained soils and is of little importance in the clayey soils of the Piedmont section and in other clayey soils.

2. In Arkansas, little or no wilt commonly appears before early June, the disease developing slowly during June and finds its maximum development in July and early August. More resistant varieties have a longer period of incubation and the greatest amount of wilt appears in them later in the season.

3. Under controlled soil conditions, using Wisconsin Soil Temperature Tanks in the greenhouse, Trice cotton is affected with wilt most severely when soil temperatures are near 30.5 C., but temperatures from 28 C. to 32 C. are very favorable to wilt development. The incidence of wilt falls off rapidly above 33 C. and above 35.5°C. little wilt occurs. No wilt has ever been observed above 37.5°C. and only one plant out of more than a hundred used developed wilt at 37.5 C. and then only after 80 days exposure to the disease. The amount of wilt decreases gradually below 28 C., but small amounts occur at 18 C., the lowest temperature employed. Serious cotton wilt infections probably will not occur in the field at soil temperatures below 23°C. except in especially susceptible varieties.

4. Soil temperatures have a very marked effect on the period of incubation of cotton wilt. At 28 to 35.5 C. cotton wilt appeared in Trice cotton in less than 12 days and the greatest amount of wilting took place within the first 24 days. Below 28 C. the incubation period was progressively longer so that at 20.5°C. only 5 per cent of wilt had appeared at the end of 24 days and at 18 C. only a trace of wilt appeared before the 36th day.

5. The effect of soil temperature upon the development of cotton wilt is closely correlated with the effect of temperature upon the growth of the cotton wilt fungus *in vitro*. The optimum temperature for the development of cotton wilt is perhaps slightly higher than the optimum temperature for the development of the cotton wilt fungus. A rapid falling off both in the development of cotton wilt and in the rate of growth of the pathogene occurs above 28 to 32 C., and the maximum temperature both for the development of cotton wilt and for the growth of the pathogene is around 37.5 -38 C. Below 30.5 C. the rate of growth of *F. vasinfectum* falls off slowly, growth still resulting at 10 C. The development of cotton wilt at lower soil temperatures likewise falls off gradually but the actual minimum was not determined. Wilt development at 18 C., however, is very slight.

6. Air temperatures as low as 24°C. may check considerably the rate of development of *F. vasinfectum* in the stems of the cotton plant even though soil temperatures are favorable for its development in the roots. Under these conditions the whole vascular system of the roots may be markedly affected and discolored without internal darkening of the stems or apparent injury to the aerial parts.

7. While the most favorable soil temperatures for the development of cotton wilt are slightly below those determined by others as most favorable for the development of the cotton plant, they fall within the range which is not at all unfavorable to its development. Sufficient data is not at hand to suggest any close correlation between vigor and lack of vigor of the host and the development of cotton wilt.

8. Thorough mixing of comparatively large amounts of the cotton wilt pathogene in pure culture with soil results in a greatly increased incidence of cotton wilt as compared to the addition of inoculum to the upper two or three inches of soil only, the rapid growth of young cotton roots through a shallow inoculated zone, apparently enabling the plant in many cases to escape infection.

9. Under field conditions at Galloway, Arkansas, in 1926, and at Marianna, Arkansas, in 1927, records of air temperatures suggest very strongly that the failure of appreciable amounts of wilt to appear even in extremely susceptible varieties for more than a month after planting is due to the inhibiting effects of low soil and air temperatures rather than to any lack of susceptibility in cotton seedlings, and that in all probability soil temperatures were most favorable to the development of wilt during July and August, the period when most of the cotton wilt in Arkansas appears.

10. The increased severity of cotton wilt in well drained, extremely sandy soils, the close correlation between the optimal temperatures for growth of *Fusarium vasinfectum* in pure culture and the optimal soil temperature for the development of cotton wilt and the pronounced effects of increased amounts of inoculum in contact with cotton roots in increasing the incidence of wilt all point to a close relationship between the conditions which bring about rapid saprophytic development of the pathogene in the soil and the development of cotton wilt. It is suggested that under natural conditions warm, well aerated sandy soils with sufficient amounts of organic matter for the development of the pathogene may allow a rapid spread of the pathogene through the soil and thus favor the attack on cotton roots.

11. The work of Balls, Richards, Park, Massey and Godfrey indicates that soreshin and related phenomena and cotyledonary infections by the angular leaf-spot pathogene in the soil are favored by the cooler soil temperatures most prevalent in the Southern United States at the time of planting cotton and that root-knot infection is possible under a wide range of soil temperatures. The work herein reported indicates that cotton wilt on the other hand, is most favored by a range of warm soil temperatures not commonly encountered in Arkansas, at least until summer is well under way.

12. Since soil temperatures of the spring months are unfavorable to the development of cotton wilt, it follows that varieties which germinate and grow better in cool soil may have more chances of escaping wilt infection than have varieties which are late maturing and not cold resistant. The further need of considering resistance to low temperatures in conjunction with wilt resistance when breeding cotton is, therefore suggested.

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